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Cobalt chloride induces neuronal differentiation of human mesenchymal stem cells through upregulation of microRNA-124a

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ABSTRACT

Human mesenchymal stem cells (hMSCs) are known to have the capacity to differentiate into various cell types, including neurons. To examine our hypothesis that miRNA was involved in neuronal differentiation of hMSCs, $CoCl_2$, a hypoxia-mimicking agent was used to induce neuronal differentiation, which was assessed by determining the expression of neuronal markers such as nestin and Tuj1. Treatment of hMSCs with $CoCl_2$ led to increased expression of miR-124a, a neuron-specific miRNA. HIF-1α silencing and JNK inhibition abolished $CoCl_2$-induced miR-124a expression, suggesting that JNK and HIF-1α signals were required for the miR-124a expression induced by $CoCl_2$ in hMSCs. Overexpression of miR-124a or $CoCl_2$ treatment suppressed the expression of anti-neural proteins such as SCP1 and SOX9. Silencing of both SCP1 and SOX9 induced neuronal differentiation of hMSCs, indicating that suppression of miR-124a targets is important for $CoCl_2$-induced neuronal differentiation of hMSCs. Knockdown of HIF-1α or inhibition of JNK restored the expression of SCP1 and SOX9 in $CoCl_2$-treated cells. Inhibition of miR-124a blocked $CoCl_2$-induced suppression of SCP1 and SOX9 and abolished $CoCl_2$-induced neuronal differentiation of hMSCs. Taken together, we demonstrate that miR-124a is critically regulates $CoCl_2$-induced neuronal differentiation of hMSCs by suppressing the expression of SCP1 and SOX9.

1. Introduction

Mesenchymal stem cells (hMSCs) are also termed as stromal cells and isolated from a variety of tissues including bone marrow and adipose tissues. Owing to their capacity to differentiate into diverse cell types, including neuronal cells, hMSCs have been used in cell-based therapies to treat neurodegenerative diseases such as cerebral ischemia [1,2]. Recent reports indicate that hMSCs not only replace damaged neurons, but also supply protective neurotropic factors [3–5]. Hypoxia play pivotal roles in maintaining homeostasis and in regulating stem cell pluripotency [6]. Under hypoxic conditions, functions of hMSCs are regulated by several transcriptional factors, including the hypoxia-inducible factors (HIFs). Hypoxia-inducible factor-1α (HIF-1α) is the key regulator of cellular response to hypoxia by activating transcription of various genes involved in cellular metabolism, angiogenesis, metastasis/invasion and apoptosis. Cobalt chloride ($CoCl_2$) induces biochemical and molecular responses similar to those observed under hypoxic conditions; for example, treatment of PC12 cells with $CoCl_2$ leads to neurite outgrowth [7].

MicroRNAs (miRNAs) are small, single-stranded RNA molecules of 21–23 nucleotides in length. They fully or partially bind to their target mRNA and post-transcriptionally regulate their target genes by inducing decay of target mRNA or suppressing translation. Function of miRNA is essential for neural development and differentiation [8–10]. MiR-124a is preferentially expressed in neurons and is upregulated during neurogenesis. It has been recently found that miR-124a positively modulates the transitory progression of adult SVZ neurogenesis by repressing Sox9 [11]. Furthermore, miR-124a promotes neuronal differentiation by regulating nervous system-specific alternative splicing [12]. However, the potential role of miR-124a in neuronal differentiation of hMSCs has not been examined.

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In the study reported here, we examined the role of miRNAs in the neuronal differentiation of hMSCs. When treated with $CoCl_2$, hMSCs differentiated into neuron-like cells. These differentiated cells expressed neuronal markers such as such as doublecortin (DCX) and Tuj1. Treatment of hMSCs with $CoCl_2$ increased miR-124a expression in a HIF-1α and JNK-dependent manner. We demonstrate that miR-124a induces neuronal differentiation of hMSCs by suppressing SCP1 and SOX9. Furthermore, anti-miR-124a abolished $CoCl_2$-induced neuronal differentiation of hMSCs. Our results demonstrate for the first time that miR-124a is critical for the $CoCl_2$-induced neuronal differentiation of hMSCs.

2. Materials and methods

2.1. Cell culture and transfection

Human mesenchymal stem cells (hMSC, Lonza) were maintained at 37 °C, and 5% CO_2 in Mesenchymal Stem Cell Growth Medium (MSCGM, Lonza) supplemented with MSCGM SingleQuots (Lonza). All experiments were performed using cells of passages 5–8. Cells were passaged when the cultures reached 90% confluence and either used for experiments or redistributed to new culture plates. Cobalt chloride ($CoCl_2$) was from Sigma–Aldrich and JNK-specific inhibitor, SP600125 was purchased from Calbiochem (La Jolla, CA). For transfection, hMSCs were plated at a density of 5×10^5 cells/dish and were transfected with control or indicated siRNAs using Lipofectamine2000 (Invitrogen) according to the manufacturer's protocol. The siRNAs targeting HIF-1α(sc-44225), SCP1 (sc-37642), and SOX9 (sc-36533) were purchased from Santa Cruz Biotechnology. Control siRNA was synthesized by Genolution. Precursor miRNA-124a (pre-miR-124a) and antisense miR-124a (anti-miR-124a) were purchased from Ambion, and were respectively used for activation or inhibition of miRNA function.

2.2. Western blot analysis

For Western blot analysis, hMSCs were lysed with RIPA buffer containing protease inhibitors and phosphatase inhibitors (Roche). Protein concentrations were determined by Bradford assay. Lysates were subjected to SDS–polyacrylamide gels electrophoresis and transferred to PVDF membranes (Millipore). Membranes were blocked by incubating overnight with 5% skim milk. Following incubation with the appropriate primary and secondary antibodies, bands were visualized with an enhanced chemiluminescence (ECL, Amersham). Antibodies for SCP1 and SOX9 were purchased from R&D Systems. Antibody for β-actin was obtained from Santa Cruz.

2.3. Quantitative real-time PCR (RT-qPCR)

To determine the level of mRNA and miRNA, total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized using Superscript III Reverse Transcriptase (Invitrogen) and RT-qPCR was performed using power SYBR® Green PCR Master Mix (Applied Biosystems). The primers used in this study were as follows: *SCP1*, forward (5′-AAG CCG GGG CAT CCT CCA CT-3′) and reverse (5′-CTG GGC CTT GGC CTC AGG GA-3′); *SOX9*, forward (5′-CCC TTC GTG GAG GAG GCG GA-3′) and reverse (5′-GGC CTG CAG CGC CTT GAA GA-3′); and *β-actin*, forward (5′-TAA GGA GAA GCT GTG CTA CG-3′) and reverse (5′-TGA AGG TAG TTT CGT GGA TG-3′). β-Actin mRNA was used for normalization. The level of miR-124a was determined using stem loop-specific RT primer and TaqMan PCR Master Mix (Applied Biosystems) and was normalized against the level of U6 snRNA.

2.4. Immunohistochemical analysis

The expression level of neuronal markers, nestin and Tuj1, was determined by immunohistochemical analysis. Briefly, hMSCs were washed with PBS, fixed in 4% paraformaldehyde for 15 min, and permeabilized with PBS containing 0.2% Triton X-100 for 10 min. After blocking with 2% bovine serum albumin, they were incubated with anti-nestin or anti-Tuj1 antibody for 2 h and then incubated with Alexa Fluor® 568-conjugated anti-mouse secondary antibody (red, for nestin) or Alexa Fluor® 488-conjugated anti-rabbit secondary antibody (green, for Tuj1) (Molecular Probes) for 1 h. Nuclei were stained by applying DAPI for 30 min. The fluorescence was visualized under a confocal microscope.

2.5. Statistical analysis

Statistically significance ($P < 0.05$) of the result was analyzed using independent-sample *t*-test. Data are expressed as mean ± SEM, and represents that from 3 to 5 independent experiments.

3. Results

3.1. $CoCl_2$ induced neuronal differentiation of human mesenchymal stem cells

Human mesenchymal stem cells (hMSCs) are multipotent stromal cells having a capacity to differentiate into various cell types including neuron. Tuj1 has been known as neuron-specific class III β-tubulin and DCX is reported to be expressed by neuronal progenitor cells or immature neuron. To investigate whether treatment of $CoCl_2$ induces neuronal differentiation, hMSCs were cultured in presence of 100 μM $CoCl_2$. After 72 h incubation, morphology of hMSCs was examined under a microscope and expression of neuronal markers such as doublecortin (DCX) and Tuj1 was analyzed by Western blot. hMSCs treated with $CoCl_2$ displayed neuron-like morphological characteristics including neurite outgrowth. Treatment of hMSCs with $CoCl_2$ dramatically induced the expression of HIF-1α and neuronal markers such as DCX and Tuj1 (Fig. 1A), suggesting that hMSCs differentiated into neuronal cells under hypoxic condition. Immunohistochemical analysis confirmed that $CoCl_2$ treatment induced neuronal differentiation of hMSCs (Fig. 1B).

3.2. $CoCl_2$ increased expression of miR-124a through HIF-1α and JNK signals

Expression of miRNAs is tightly controlled during neural development, and is essential for neuronal differentiation. To examine if miRNAs were involved in $CoCl_2$-induced neuronal differentiation, hMSCs were treated with $CoCl_2$ and the expression of miR-124a was analyzed. As expected, treatment of hMSCs with $CoCl_2$ induced HIF-1α expression within 3 h. In company with HIF-1α induction, the level of miR-124a was significantly increased in $CoCl_2$-treated hMSCs (Fig. 1C). MiR-124a is known to be preferentially expressed in neurons and is upregulated during neurogenesis. It was also reported to promote neuronal differentiation by suppressing the expression of PTBP, a global repressor of alternative splicing, which triggers brain-specific alternative pre-mRNA splicing [12]. To elucidate the detail mechanism, the role of HIF-1α and JNK signal in $CoCl_2$-induced miR-124a expression was investigated. First, we examined whether HIF-1α induction was involved in $CoCl_2$-induced miR-124a expression. hMSCs were transfected with control (CTRL) or HIF-1α siRNA; 48 h post-transfection, cells were treated with DMSO or $CoCl_2$ for 3 h. The level of HIF-1α and miR-124a was determined by Western blot and RT-qPCR, respectively. Treatment

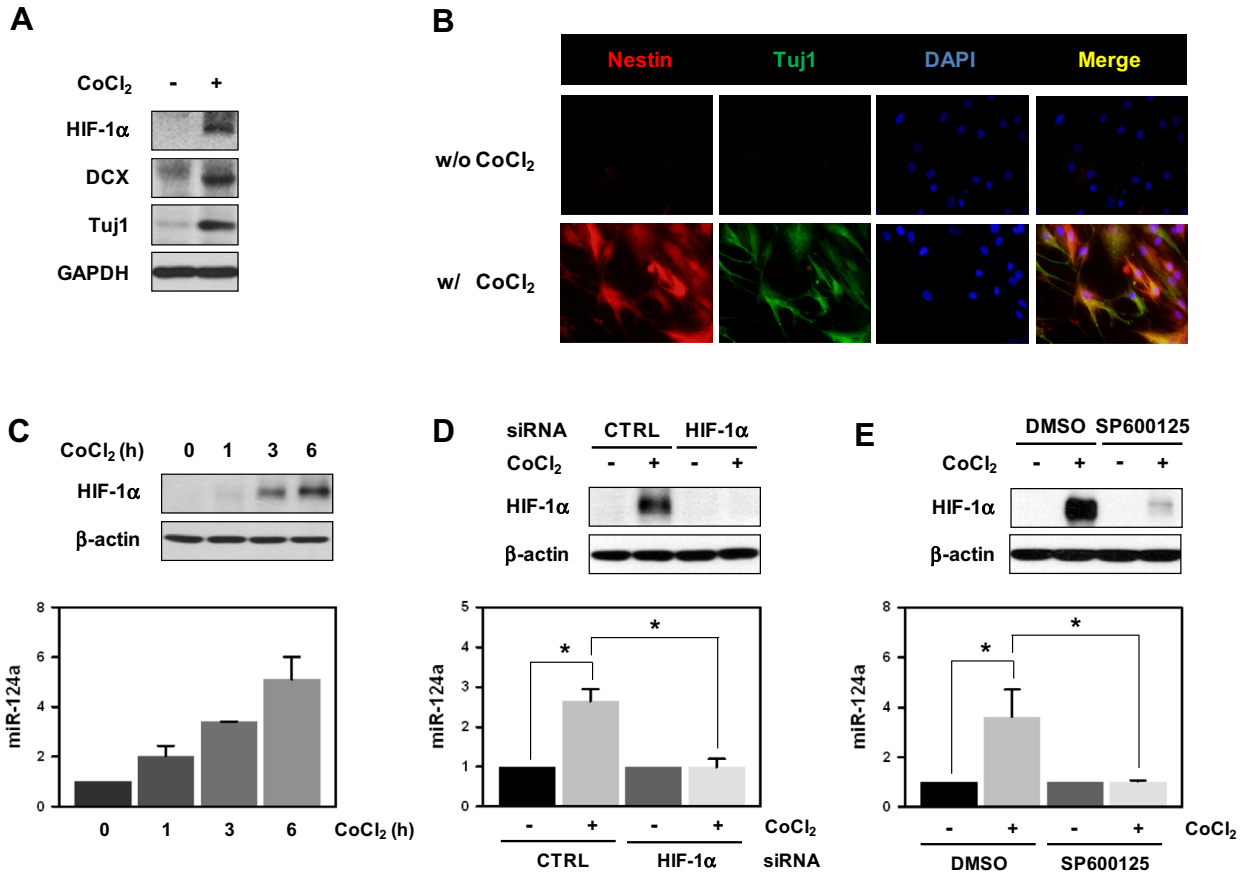

Fig. 1. $CoCl_2$ induces neuronal differentiation of hMSCs through HIF-1α- and JNK-dependent expression of miR-124a. (A) To examine if $CoCl_2$ treatment induced neuronal differentiation of hMSCs, hMSCs were treated with 100 μM $CoCl_2$ for 72 h. Whole cell lysates were prepared and the expression level of HIF-1α, doublecortin (DCX), and Tuj1 were analyzed by Western blot. (B) Neuronal differentiation of hMSCs was assessed by determining the expression of neuronal markers such as nestin (red) and Tuj1 (green) by immunohistochemical analysis. (C) hMSCs were treated with 100 μM $CoCl_2$ and then harvested at indicated time. The expression level of HIF-1α and miR-124a was determined by Western blot and RT-qPCR, respectively. (D) hMSCs were transfected with control siRNA (CTRL) or HIF-1α-specific siRNA (HIF-1α); 48 h post-transfection, hMSCs were treated with 100 μM $CoCl_2$ for 3 h. The expression of HIF-1α and miR-124a was determined by Western blot and RT-qPCR, respectively. (E) hMSCs were pretreated with vehicle control (DMSO) or JNK-specific inhibitor, SP600125, for 1 h and sequentially treated with 100 μM $CoCl_2$ for 3 h. The expression level of HIF-1α and miR-124a was determined by Western blot and RT-qPCR, respectively. All data represent mean ± SD ($n = 3$). Asterisk (*) indicates statistical significance of $P < 0.05$ as determined by Student's *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of hMSCs with $CoCl_2$ increased miR-124a expression and silencing of HIF-1α significantly inhibited $CoCl_2$-induced miR124a expression (Fig. 1D). These results suggested that $CoCl_2$-induced miR-124a expression in hMSCs was HIF-1α-dependent. Since $CoCl_2$ is known to activate JNK, the effect of JNK inhibition on miR-124a expression was examined. For this, hMSCs were pretreated with a JNK-specific inhibitor, SP600125 for 1 h and then incubated with $CoCl_2$ for 3 h. Inhibition of JNK by SP600125 blocked $CoCl_2$-induced expression of HIF-1α and miR-124a expression as well (Fig. 1E). From above results, we found that treatment of $CoCl_2$ induced miR-124a expression through HIF-1α- and JNK-dependent pathway in hMSCs.

3.3. $CoCl_2$-induced of miR-124a suppressed the expression of SCP1 and SOX9

Anti-neural protein, SCP1 (small C-terminal domain phosphatase 1) is generally expressed in non-neuronal cells and closely associated with neurogenesis. MiR-124a is known to regulate neurite outgrowth during neuronal differentiation [13] and to induce neurogenesis of P19 cell by suppressing the expression of SCP1 [14]. In P19 cells, a phosphatase-inactive SCP1 mutant promotes neurogenesis by interfering with the function of REST, suggesting that downregulation of REST/SCP1 pathway is critical for neurogenesis [14]. It was also reported that miR-124a regulates adult neurogenesis in the subventricular zone (SVZ) stem cell lineage by directly suppressing SOX9 expression [11]. To examine if miR-124a suppressed the expression of anti-neural proteins, including SCP1 and SOX9, hMSCs were transfected with pre-miR-124a; 48 h post-transfection, the expression of SCP1 and SOX9 was analyzed by Western blot. Overexpression of miR-124a led to decreased expression of SCP1 and SOX9 transcripts as well as protein (Fig. 2A). Since, as shown in Fig. 1C, treatment of $CoCl_2$ induced miR-124a expression, the effect of $CoCl_2$ on SCP1 and SOX9 expression in hMSCs was examined. Similar to that found following the overexpression of miR-124a, treatment of hMSCs with $CoCl_2$ led to decreased expression SCP1 and SOX9 transcripts as well as protein (Fig. 2B). To determine if miR-124a-mediated suppression of SCP1 and SOX9 expression was required for $CoCl_2$-indued neuronal differentiation, hMSCs were transfected with CTRL siRNA or siRNA targeting SCP1 or SOX9. Forty-eight hours after transfection, the expression levels of nestin and Tuj1 were analyzed by immunohistochemistry. Transfected hMSCs showed decreased expression of SCP1 and SOX9 protein and mRNA as well (Fig. 2C). To determine the role of SCP1 and SOX9 in neuronal differentiation, hMSCs were transfected with SCP1- or SOX9-specific siRNA; 48 h post-transfection, the level of nestin and Tuj1 was determined by immunohistochemical analysis. As shown in Fig. 4D, knockdown

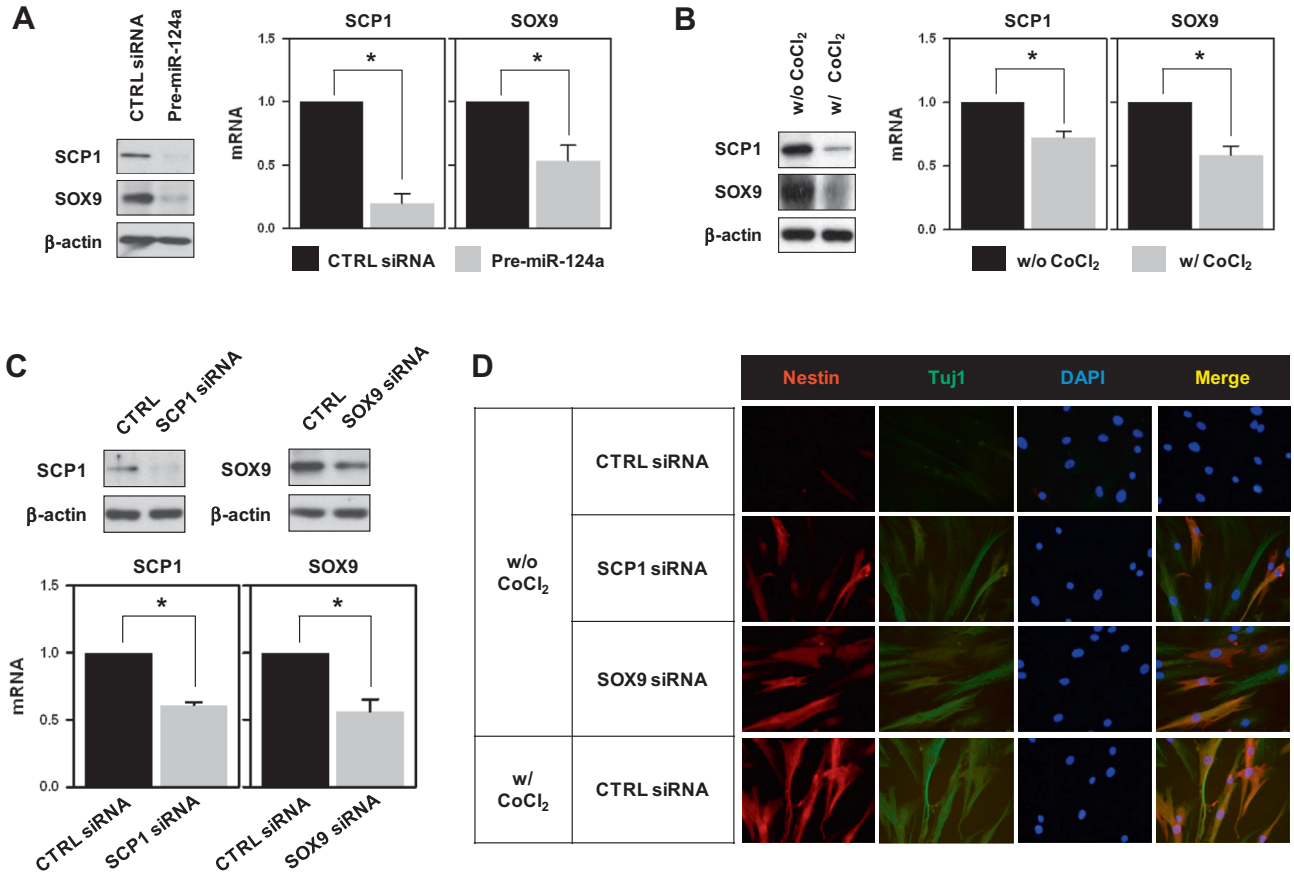

Fig. 2. Suppression of anti-neural SCP1 and SOX9 by miR-124a is responsible for $CoCl_2$-induced neuronal differentiation of hMSCs. (A) To investigate the mechanism by which $CoCl_2$-induced neuronal differentiation of hMSCs, hMSCs were transfected with pre-miR-124a; 48 h post-transfection, whole cell lysates and total RNA were prepared as described in Section 2. The protein and mRNA level of SCP1 and SOX9 were determined by Western blot and RT-qPCR, respectively. (B) After hMSCs were treated with 100 μM $CoCl_2$ for 3 h, whole cell lysates and total RNA were prepared. The protein and mRNA level of SCP1 and SOX9 was determined by Western blot and RT-qPCR, respectively. All data represent mean ± SD ($n = 3$). Asterisk (*) indicates statistical significance of $P < 0.05$ by Student's t-test. (C) To investigate whether suppression of SCP1 and SOX9 is responsible for $CoCl_2$-induced neuronal differentiation of hMSCs, hMSCs were transfected with control, SCP1, or SOX9 siRNA; 48 h post-transfection, whole cell lysates and total RNA were prepared for determining the level of SCP1 and SOX9 protein and mRNA, respectively. (D), Effect of SCP1 or SOX9 silencing on neuronal differentiation of hMSCs was assessed by immunochemical analysis of the expression of nestin (red) and Tuj1 (green). Data represent mean ± SD ($n = 3$). Asterisk (*) indicates statistical significance of $P < 0.05$ as determined by Student's t-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of SCP1 or SOX9 increased the expression of nestin and Tuj1 in hMSCs, indicating that SCP1 and SOX9 were involved in $CoCl_2$-induced neuronal differentiation of hMSCs. Therefore, $CoCl_2$-induced expression of miR-124a triggered neuronal differentiation of hMSCs through downregulation of anti-neural SCP1 and SOX9.

3.4. HIF-1α silencing and JNK inhibition blocked $CoCl_2$-induced downregulation of SCP1 and SOX9

As shown in Fig. 1D and E, $CoCl_2$-induced expression of miR-124a was blocked by HIF-1α silencing or JNK inhibition. To investigate whether the expression of SCP1 and SOX9 was restored by HIF-1α silencing or JNK inhibition, hMSCs were transfected with CTRL siRNA or HIF-1α siRNA, and then treated with DMSO or $CoCl_2$ for 3 h. The expression levels of SCP1 and SOX9 protein and mRNA were determined by Western blot and RT-qPCR, respectively. Silencing of HIF-1α reversed $CoCl_2$-induced downregulation of SCP1 and SOX9 (Fig. 3A–C). Pretreatment of hMSCs with SP600125 for 1 h blocked $CoCl_2$-induced decrease in SCP1 and SOX9 (Fig. 3D–F). These results indicated that $CoCl_2$-induced expression of miR-124a and neuronal differentiation of hMSCs occurred through HIF-1α- and JNK-dependent pathway.

3.5. Inhibition of miR-124a abolished $CoCl_2$-induced neuronal differentiation of hMSCs

We found that $CoCl_2$ increased miR-124a expression in hMSCs via HIF-1α/JNK pathway, leading to a reduction in the expression of SCP1 and SOX9. To examine if miR-124a expression was critical for $CoCl_2$-induced neuronal differentiation, hMSCs were transfected with anti-miR-124a. Forty-eight hours after transfection, cells were treated with $CoCl_2$ for 3 h. Suppression of miR-124a by anti-miR-124a restored the expression of SCP1 and SOX9 in hMSCs (Fig. 4A). To further analyze the effect of anti-miR-124a on $CoCl_2$-mediated neuronal differentiation, hMSCs were transfected with CTRL siRNA, pre-miR-124a or anti-miR-124a; 48 h post-transfection, hMSCs were treated with DMSO or $CoCl_2$ for 72 h. As shown previously, hMSCs treated with $CoCl_2$ showed increased expression of neuronal markers such as nestin and Tuj1. However, no such increase in nestin and Tuj1 were found in anti-miR-124a-trasfected hMSCs (Fig. 4B). Furthermore, hMSCs transfected with pre-miR-124a showed increased expression of nestin and Tuj1 (Fig. 4B). These results suggested that miR-124a plays a critical role in $CoCl_2$-induced neuronal differentiation of hMSCs. The results are summarized in Fig. 4C.

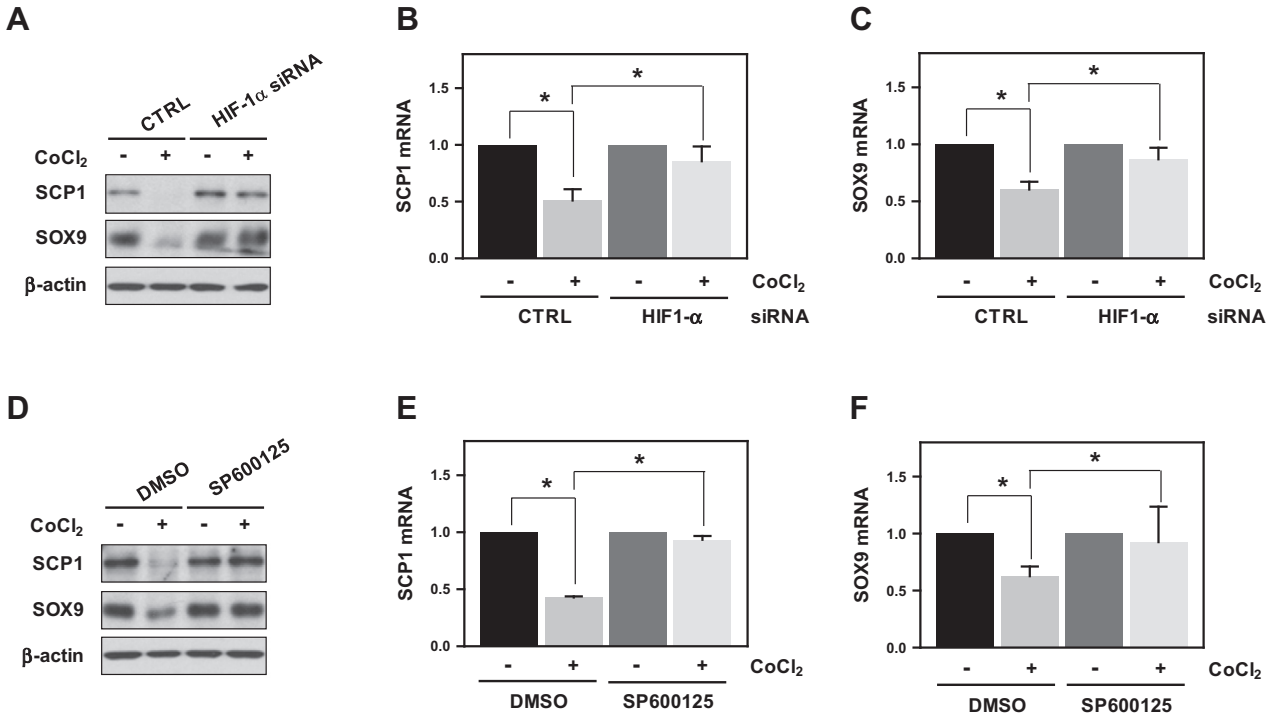

Fig. 3. $CoCl_2$-induced downregulation of SCP1 and SOX9 is reversed by HIF-1α silencing or JNK inhibition. (A–C) Knockdown of HIF-1α was achieved by transfecting hMSCs with control (CTRL) or HIF-1α-specific siRNA as described above. Transfected hMSCs were treated with $CoCl_2$ for 3 h and whole cell lysates and total RNA were prepared for determining the level of SCP1 and SOX9 protein and mRNA, respectively. (D–F) To inhibit JNK activation, hMSCs were pretreated with 10 μM SP600125 for 1 h and then treated with $CoCl_2$ for 3 h. The protein and mRNA level of SCP1 and SOX9 were determined by Western blot and RT-qPCR, respectively. All data represent mean ± SD ($n = 3$). Asterisk (*) indicates statistical significance of $P < 0.05$ as determined by Student's *t*-test.

4. Discussion

The multilineage differentiation potential of hMSCs has allowed their application in cell-based therapies for neurodegenerative diseases such as stroke. It was reported that the beneficial effects of hMSCs in the treatment of neurodegenerative diseases were primarily due to their neuro-protective activities. Moreover, hMSCs are known to have the capacity to differentiate into neuronal cells. Retinoic acid (RA) and brain-derived neurotropic factor (BDNF) induce the differentiation of bone marrow stromal cells (BMSCs) into neural cells [15]. Antioxidants such as β-mercaptoethanol (BME), dimethyl sulfoxide (DMSO), and butylated hydroxyanisole (BHA), have been shown to induce neuronal differentiation of bone marrow-derived mesenchymal cells (BM-MSCs) [16]. We hypothesized that miRNAs could control the processes governing neuronal differentiation of hMSCs. To examine this possibility, we analyzed the expression of miR-124a during $CoCl_2$-induced neuronal differentiation of hMSCs. Treatment of hMSCs with $CoCl_2$ displayed the morphological and molecular characteristics of neuronal differentiation. We found that neuronal differentiation of hMSCs occurred through $CoCl_2$-induced, HIF-1α- and JNK-dependent miR-124a expression, which suppressed the expression of anti-neural SCP1 and SOX9. Accordingly, inhibition of miR-124a restored the expression of SCP1 and SOX9, and abolished $CoCl_2$-induced neuronal differentiation of hMSCs.

The miR-124a, a neuron-specific miRNA, is one of most abundantly expressed miRNAs in brain [17] and is firstly identified in differentiating neurons [18]. The miR-124a plays important roles in neuronal development and its expression level is maintained during neuronal maturation [19]. In mouse P19 cells, overexpression of miR-124a promotes neurite outgrowth [13]. Recent studies have shown that miR-124a promotes neuronal differentiation through downregulation of SCP1 and PTBP1. During CNS development, miR-124a regulates neurogenesis by timely suppressing the expression of SCP1 [14]. In non-neuronal cells, REST/NRSF suppresses the expression of neuron-specific genes by binding to a conserved repressor element (RE1). Similar to REST, SCP1 is also anti-neural protein and expressed in non-neuronal cells. REST brings SCP1 to RE1-containing neural genes, thereby suppressing the expression of genes required for neurogenesis. It was recently demonstrated that miR-124a positively modulates the transitory progression of adult SVZ neurogenesis by repressing SOX9, a SRY-box transcriptional factor [11]. SOX9 induction by sonic hedgehog is necessary for the maintenance of stemness in neural stem cells [20]. Therefore, suppression of SCP1 by miR-124a triggers processes that allow neuronal gene expression and neuronal differentiation. MiR-124a has also been found to directly target PTBP1 which is a repressor of alternative splicing in non-neuronal cells [12]. During neuronal differentiation, miR-124a suppresses PTBP1, resulting in alternative splicing of PTBP2 mRNA. Correctly spliced PTBP2 mRNA leads to increased expression of PTBP2 by blocking nonsense-mediated decay (NMD). In addition to miR-124a, miR-125b are found to induce neuronal differentiation of neuroblastoma SH-SY5Y cells [21]. Overexpression of either miR-124a or miR-125 was found to increase the number of differentiated SH-SY5Y cells.

Recent studies have demonstrated that oxygen-dependent gene expression plays important roles in proliferation, survival, and differentiation. Among the various factors associated with oxygen-dependent gene expression, HIF-1α has been found to promote neurogenesis *in vitro* and *in vivo*. It was also reported that $CoCl_2$ and ROCK inhibitor synergistically induces differentiation of mesenchymal stem cell into neuron-like cells [22]. Several miRNAs are reported to be regulated by HIF-1α, which are termed as hypoxia-regulated miRNAs (HRMs). Kulshreshtha et al. identified specific spectrum of miRNAs that are upregulated by hypoxia;

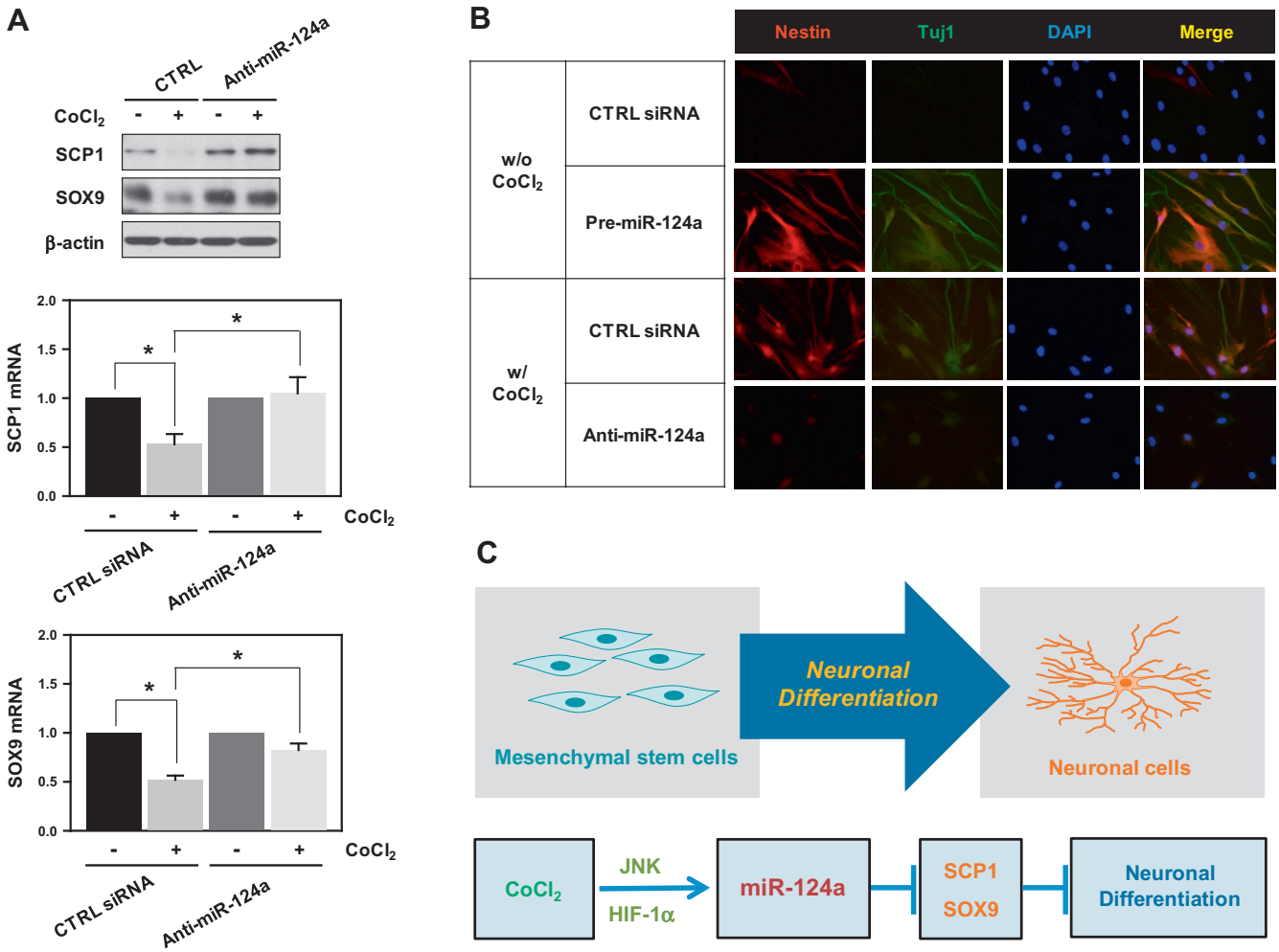

Fig. 4. Anti-miR-124a abolishes $CoCl_2$-induced neuronal differentiation of hMSCs by reestablishing the expression of SCP1 and SOX9. (A) Inhibition of miR-124a was achieved by transfecting hMSCs with control (CTRL) or anti-miR-124a as described in Section 2. Transfected hMSCs were treated with $CoCl_2$ for 3 h and whole cell lysates and total RNA were prepared for determining the level of SCP1 and SOX9 protein and mRNA, respectively. All data represent mean ± SD (n = 3). Asterisk (*) indicates statistical significance of $P < 0.05$ as determined by Student's t-test. (B) hMSCs were transfected with control (CTRL), pre-miR-124a, or anti-miR-124a; 48 h post-transfection, hMSCs were treated with solvent control (DMSO) or $CoCl_2$ for 72 h. $CoCl_2$-induced neuronal differentiation of hMSCs was assessed by immunochemical analysis of the expression of nestin (red) and Tuj1 (green). (C) Schematic model of the function of miR-124a in $CoCl_2$-induced neuronal differentiation of hMSCs was illustrated. Briefly, $CoCl_2$ treatment induces neuronal differentiation of hMSCs by increasing expression of miR-124a, which results in downregulation of anti-neural SCP1 and SOX9. More details are mentioned in text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

miR-23, -24, -26, -27, -103, -107, -181, -210, and -213 are induced by low level of oxygen, at least some through HIF-1α-dependent way [23]. In human endothelial cells, HIF-1α upregulates miR-424 and promotes angiogenesis by targeting cullin 2 (CUL2) which is a critical scaffold protein for ubiquitin ligase system [24]. The level of miR-424 is controlled by PU.1-dependent transactivation which is increased in hypoxia-stressed endothelium. It suggests that HIF-1α-regulated miR-424 is an important factor for angiogenesis in post-ischemic condition [24]. The precise mechanisms by which $CoCl_2$-induced expression of HIF-1α increases cellular level of miR-124a expression in hMSCs remain to be identified. Our results demonstrate that the critical role of miR-124a in $CoCl_2$-induced neuronal differentiation of hMSCs.

Acknowledgments

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